Hot flushes and reproductive hormone levels during the menopausal transition

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Pages: 19 pages text, including 5 pages bibliography; 1 figure, 3 tables

Abbreviated title: Hot flushes and reproductive hormone levels

Abstract:

Introduction: Evidence suggests that hot flushes are associated with fluctuating oestradiol (E2) during menopause, as well as changes in follicle-stimulating hormone (FSH) and inhibin B. The relationship between hot flushes and anti-Müllerian hormone (AMH) is unknown.

Aim: To examine specific hormone levels and ethnic differences in relation to hot flushes.

Methods: Data were drawn from 108 women aged 40-59 years from three groups: European women in London, UK; Bangladeshi women in Sylhet, Bangladesh, and Bangladeshi women who migrated to London as adults. Symptom information was collected via questionnaires. Serum blood samples were collected to detect inhibin B, AMH, FSH, and E2.

Results: AMH and FSH were significantly associated with hot flush occurrence in the past 2 weeks. Inhibin B and E2 were not associated with hot flushes. Body mass index (BMI), ethnicity, and education level were associated with both hot flush occurrence and frequency. Menopausal status was also associated with hot flush frequency.

Conclusion: Relationships between AMH, FSH and symptoms are indicative of women's progress through the menopausal transition. The influence of BMI and education suggest that lifestyle changes may contribute to the management of symptoms. This merits further research.

Key words: menopause, reproductive hormones, vasomotor symptoms, hot flushes, hot flashes, ethnicity

Introduction

The menopausal transition is marked by significant changes in reproductive hormone levels with an eventual decline in levels of reproductive steroids and an increase in gonadotrophins [1-4]. Hormonal changes can be observed as early as the third decade of life for most women, emphasizing the processual nature of reproductive ageing [5-7]. Menopause is accompanied by a suite of symptoms that vary in occurrence and severity among and between women, with hot flushes being among the most troublesome [4,8]; up to 80% of women experience hot flushes in the U.S. and Britain [9,10]. Hot flushes are associated with declining levels of oestrogen [11], purportedly because a decline in this hormone is associated with a narrowing of the thermoneutral zone between the core body temperatures that trigger shivering and sweating [12]. When the sweating threshold is lowered by hormonal changes, women sweat more easily in response to increases in core body temperature [13].

Understanding the relationship between hot flushes and hormonal changes is complicated by a lack of consistency in the results found in several studies. Most theories have concentrated on oestrogen rather than any other hormone because of its known role in thermoregulation [11,13]. Even here, however, the picture is far from clear. For example, oestrogen levels do not necessarily differ significantly between asymptomatic and symptomatic women and are not predictive of symptoms during the climacteric [14,15]. Hot flushes are also closely timed with luteinising hormone (LH) pulses, which might suggest that they are related in some way to gonadotrophin-releasing hormone (GnRH) synthesis. Rance et al. [16] suggest that hot flushes are caused by the activation of kisspeptin/neurokinin B/dynorphin (KNDy) neurons following post-menopausal oestrogen withdrawal because these neurons modulate GnRH synthesis, express oestrogen receptors, and are hypertrophied in post-menopausal women. Such theories need further testing.

Many studies have demonstrated variation in age at menopause as well as differences in the experience of menopausal symptoms among women of different ethnicities [8,17,18].

Median ages at menopause range from 47 to 53 years among different populations, most likely due to significant environmental variation between populations in, for example, nutritional intake and exposure to diseases during childhood, energy expenditure, parity, and smoking rates [19]. There also appear to be differences in menopausal age because of allelic variations [20-22]. We have previously reported that women of Bangladeshi origin – those still living in Bangladesh and migrant women who moved to London during adulthood – had significantly earlier ages at menopause compared to London women of European origin [23]. These data are confirmed by Begum et al [24] who found that age-matched Bangladeshi sedentees and adult migrants had levels of inhibin B, AMH, FSH, and E_2 that demonstrated an earlier age-related decline in ovarian reserve when compared to women of European origin.

There is a large range of individual and population variation in women's experience of hot flushes [25-27]. Differences in the prevalence of hot flushes have been reported by ethnicity, with African-American and Hispanic women reporting the highest frequency, and southeast Asian women reporting the lowest [9, 28-30]. In our own studies of vasomotor symptoms among Bangladeshi and European women, we have found differences in the experience of hot flushes between middle class Hindu and Muslim women living in Bangladesh that appear to be related to differences in clothing and patterns of prayer. Islamic forms of prayer involve the physical exertion of repeated bending and kneeling [31]. So far, however, we have not yet explored relationships between levels of reproductive hormones and the experience of hot flushes among the same groups of women we have previously studied. This paper therefore investigates the association between inhibin B, AMH, FSH, and E_2, and the occurrence and frequency of hot flushes among two groups of Bangladeshi women living in Bangladesh and the UK, and a comparative group of women of European origin living in London. To our knowledge, no prior papers have explored the relation between AMH and hot flushes. We hypothesized an increased probability of hot flashes and increasing hot flash frequency in association with lower levels of inhibin B, AMH, and E_2, and higher levels of FSH while controlling for other variables known to be associated with hot flashes. We also hypothesized a higher likelihood of hot flashes in the Bangladeshi populations.

METHODS

Study participants

Participants (n=108) who volunteered to give blood were drawn from a larger sample of women (n=485) recruited into a study of the menopausal transition in Bangladesh and London [23,31,32]. These included: Bangladeshi women living in Sylhet, northeast Bangladesh (sedentees), Bangladeshi women living in London (mostly in Camden and East London) who migrated to London as adults (adult migrants), and women of European origin living in London (Europeans). The subsample analysed here comprises sedentees (n=36), adult migrants (n=53), and Europeans (n=50). In Sylhet, women were recruited through personal contacts and word of mouth while, in London, Bangladeshi migrants were recruited through community centres and word of mouth. The European women in London lived in similar neighbourhoods as the migrants, and were recruited through advertisements in local newspapers and flyers advertising the study. Eligibility criteria included: ages 40-59, no exogenous hormone use (hormone therapy or contraceptives) in the last 3 months, not currently lactating or pregnant, and no previous medical history of oophorectomy, hysterectomy, thyroid disease, polycystic ovaries, or type 1 diabetes.

Because of the relatively small sample size, World Health Organization [33] criteria were used to classify women into three simple categories of menopausal status: 1) premenopausal if they had menstruated within the last 2 months, 2) peri-menopausal if they had menstruated within the last 3 to 12 months, and 3) post-menopausal if they had not menstruated in the last year. Very few (n=5) women in the sample were peri-menopausal and, for this study of hot flushes, these were combined with post-menopausal women. Height was measured with a free-standing anthropometer to the nearest 0.1 cm, and weight was measured to the nearest 0.1 kg. Body mass index (BMI) was computed as weight/height^2. Education was coded as low, medium, or high relative to the norms of the population.

Data collection

Data were collected using questionnaires administered in Bangla or English. These included information on demographics, reproductive and developmental histories, menopausal status and symptoms, education and employment status, and migration and medical histories. To assess hot flash experience, women were asked, “Thinking back over the past two weeks, have you ever been bothered by hot flushes? Please indicate the extent to which you were bothered over the past two weeks by hot flushes (not at all, a little, quite a bit, extremely.)” In addition, women were asked, “How frequently do you have hot flushes (currently): rarely (less than once/month), once/month, twice/month, once/week, twice/week, 3-4 times/week, once/day, twice/day, 3-4 times/day, 5 or more times/day.” In the Bangla language, *gorum vap laga* (feeling steamy hot) was used to describe hot flashes. Bangladeshi women also spoke a Sylheti dialect, and used the terms *groom faap laga* (feeling hot on the inside and/or queasy or suffocating), *akhata groom laga* (a sudden feeling of heat), and *matha dia dhuma jai* (smoke coming from the head) to describe hot flushes [31].

Some women were born before the standard recording of births in Bangladesh and so did not know their exact age. Bangladeshi women were asked “Do you know your exact date of birth?” Among the women who gave blood for this study, all but one migrant (97%) and almost half of the sedentees (46%) were sure of their birth date. A calendar of 21 events including the India-Pakistan war, the War of Independence, and various country-level natural disasters (cyclones and floods) were used to estimate ages for the remaining women [23].

Hormonal Analyses

Five ml blood samples for analyses of inhibin B, AMH, FSH and E_2 were taken from pre-menopausal women on days 4 to 6 of their menstrual cycle, and on any day for post-menopausal women. Sampling days were chosen to capture peak levels of inhibin B without compromising rising levels of E2 and FSH [34]; AMH levels remain relatively stable across the cycle [35]. The samples were immediately centrifuged to separate the serum and stored at -20^{o} Celsius in laboratories at the University College London (UCL) or Osmani Medical College, Sylhet. Samples stored in Sylhet were transported on dry ice and flown to UCL where all of the assays were completed. Methods for the analyses of the hormones are described elsewhere [24].

Statistical analysis

All of the hormonal data were log-transformed prior to analyses. Hot flushes experienced during the past two weeks were recoded from ordinal categorical data to binary data. 'None' was categorized as '0,' and 'a little,' 'quite a bit,' and 'extreme,' were categorized as '1.' One-way ANOVAs were performed to compare mean ages and hormone levels across groups.

Binary logistic regressions were conducted to examine hot flushes (none vs. a little/quite a bit/extreme) during the past two weeks in relation to each of the reproductive hormones where Model 1 included all the hormones, Model 2 included just inhibin B, Model 3 just AMH, Model 4 just FSH, and Model 5 just E2. Covariates included BMI, menopausal status (pre- vs. peri/post), ethnicity (European, Bangladeshi adult migrant, Bangladeshi sedentee), infectious disease load (0-2, 3-4, and 5-6 exposures), and population-specific level of education (low, medium, high).

Linear regressions were used to explain variation in hot flush frequency, from "rarely" to "5 or more times/day," examining each of the hormones together (Model A) and individually (Model B=inhibin B, Model C=AMH, Model D=FSH, and Model E=E2), and including BMI, education level, ethnicity and menopausal status as independent variables.

Both logistic and linear regressions were also run within each study group to identify variables associated with hot flushes experienced during the past 2 weeks, as well as hot flush frequency, with models that included all four hormones while controlling for BMI, education level, ethnicity and menopausal status. The analyses were also repeated for the women who were sure of their birthdate (n=84). Significance was determined using a p-value of <0.05. All data analyses were carried out using SPSS Version 22.

Ethics

Ethical approval was obtained from the Ethics Committees at University College London, Durham University's Department of Anthropology, the Osmani Medical College, Sylhet, Bangladesh and the Institutional Review Board at the University of Massachusetts, Amherst.

All women gave written, informed consent and were financially compensated for their time and participation. Data were stored in accordance with the Data Protection Act (UK).

Results

In the original sample (n=485), Europeans, migrants, and sedentees did not differ by age (mean 47.0 years, s.d. 7.1) or menopausal status (55% pre-menopausal, 8% peri-menopausal, and 37% postmenopausal). Europeans had the lowest BMI ($F_{2,479} = 8.5$, $p<0.01$). Sedentees and migrants were more likely to be married compared to Europeans ($p<0.01$), and, despite living in materially difficult circumstances, sedentees were more likely to self-report as financially "well-off" compared to Bangladeshis and Europeans living in the UK ($p<0.01$). Fewer Europeans reported any hot flashes (32%) compared to migrants (43%) and sedentees (45%, $p<0.01$).

Descriptive statistics for the participants in this study (n=108) are presented in Table 1. Mean age did not differ significantly across groups ($F_{2,105} = 0.4$, $p = 0.66$). There was a significant difference in inhibin B levels between groups ($F_{2,105} = 4.8$, $p=0.01$), with sedentees having significantly lower levels than adult migrants and European women, but there were no significant differences among groups in AMH levels ($F_{2,105} = 1.8$, $p=0.17$), FSH levels ($F_{2,105} = 1.3$, $p=0.27$), or in E_2 levels ($F_{2,105} = 0.3$, $p=0.77$). There were no significant differences in BMI between groups ($F_{2,105} = 1.3$, $p=0.28$).

[Table 1 goes about here.]

Hot flush occurrence

The experience of any hot flashes during the past two weeks differed across ethnic groups, with migrants (59%) and sedentees (57%) more likely to report any hot flash experience compared to the migrants' European neighbors (27%, $p=0.006$). Table 1 shows the percentage of women who experienced any hot flushes during the past 2 weeks by ethnic group ($p=0.002$). In the logistic regression models (Table 2), higher levels of AMH decreased the likelihood that hot flushes occurred in the past two weeks (Model 1: OR 0.14; 95% CI 0.02-0.92; Model 3: OR 0.19; 95% CI 0.05-0.69), while higher FSH levels significantly increased the probability that hot flushes occurred in the last two weeks only in the individual model (Model 4: OR 8.47, 95% CI 1.28-56.29).

[Figure 1 and Table 2 go about here.]

Ethnicity was a significant determinant of the likelihood of experiencing hot flushes in all five logistic regression models, where being a Bangladeshi woman (whether sedentee or adult migrant) increased the probability of experiencing hot flushes in the past two weeks compared to Londoners of European origin. The only exception was the logistic regression specific to inhibin B (Model 2) where only adult migrants were more likely to experience hot flushes compared to European Londoners. When analyses were repeated with only the women who were sure of their age at menopause, the number of sedentees dropped by about half, and sedentees were no longer more likely to experience hot flushes compared to European Londoners.

A higher BMI was a significant determinant of the probability of experiencing hot flushes in Model 1 (all hormones), Model 3 (AMH) and Model 4 (FSH) (Table 2). The highest relative level of education significantly decreased the likelihood of hot flush report in Models 1 (all hormones) and 3 (AMH). The highest level of education approached significance as decreasing the likelihood of hot flushes in all other models ($p<0.1$). Menopausal status increased the likelihood of hot flushes when no hormones were included in the model (OR 2.56, 95% CI 1.01-6.46; not shown), but was not a significant determinant of hot flushes after controlling for levels of hormones.

Hot flush frequency

Results from the multiple linear regressions examining the relationships between hot flush frequency and hormone levels are shown in Table 3. Hormone levels were not associated with hot flush frequency. . Ethnicity, BMI, menopausal status and education level significantly predicted hot flush frequency in all 5 linear regression models. Bangladeshi women experienced a significantly higher frequency of hot flushes; an increasing BMI was associated with more frequent hot flushes; and higher education among women was related to less frequent hot flushes. Peri/post-menopausal women experienced hot flushes more frequently compared to pre-menopausal women.

[Table 3 goes about here.]

Logistic and linear regressions were also run individually within each group to ascertain whether associations between hormones and symptoms differed on an intra-group level; sample sizes on this basis were small (sedentees: hot flush occurrence (n=16), hot flush frequency (n=12); adult migrants: hot flush occurrence (n=23), hot flush frequency (n=25); and Europeans: hot flush occurrence (n=11), hot flush frequency (n=29)). In the logistic regressions, there were no significant associations between hot flush occurrence and any of the hormones within any of the groups(not shown).

Discussion

This study set out to examine the relationship between levels of four reproductive hormones and the occurrence and frequency of hot flushes during the menopause. It also examined whether any such relationships differed between Bangladeshi women and women of European origin. While other studies have looked for relationships between hot flushes and inhibin B, FSH, and E_2 [36-39], this is the first study to our knowledge that has also explored potential associations with AMH. Relationships were indeed found between AMH, FSH, and menopausal symptoms. Ethnicity, education level, and BMI were also found to be associated with the experience of vasomotor symptoms.

In the logistic regression models for all women, AMH and FSH, but not inhibin B or $E_{2,}$ were significantly associated with the occurrence of hot flushes. Lower levels of AMH were significantly associated with the likelihood that women would experience hot flushes. AMH reflects ovarian reserve and decreases as the number of follicles declines with age [6,40]. This decrease is one of the first biological markers of menopause, and AMH levels have been discussed as a possible diagnostic blood test for the post-menopausal state [41,42]. Based on their study of 147 women aged 40-50 years in Tehran, Tehrani et al [42] suggested a threshold level of 0.39ng/ml AMH as a potential boundary marking the menopausal transition. In our sample here, AMH most likely reflects menopausal status, rather than a functional role in causing hot flushes. Because AMH levels are significantly lower in peri/post-menopausal women ($p<0.001$), this may explain why menopausal status is not associated with the likelihood of hot flushes when AMH (and other hormones) are included in the model.

Levels of FSH were positively associated with the likelihood of hot flush occurrence in our sample of all women, consistent with other findings [37-39]. FSH begins to rise approximately 6 years before the final menstrual period, and the rate of increase is highest 2 years prior to the last menstruation. Although FSH declines again post-menopause, it does not ever reach pre-menopausal levels [3]. A woman can be diagnosed as possibly post-menopausal by practitioners in the UK if her blood levels of FSH are >30IU/L [43]. According to the authors of STRAW+10, “vasomotor symptoms [are] likely” at Stage -1, and a woman’s blood FSH level will be approximately 25 IU/L or above [44]. It is possible that FSH levels have a role in explaining the mechanisms of hot flushes but again, like AMH levels, an association between FSH and hot flushes might be due the relationship between hot flushes and menopausal status.

There was no association in the whole sample of women between levels of E_2 and either the occurrence or frequency of hot flushes. Freedman [14] also found that levels of E_2 did not differ between symptomatic and non-symptomatic women, but Gold et al. [38], using longitudinal data, found that E_2 was significantly negatively associated with vasomotor symptoms. Freeman et al. [37] have also found that fluctuating levels of E_2 in individual women were positively associated with hot flush occurrence in unadjusted analysis. Since the study presented here is cross-sectional and relied on single hormone samples, we cannot mirror the latter study.

Hot flush occurrence and frequency were also significantly associated with covariates in the models, namely ethnicity, BMI and education level. In terms of ethnicity, Bangladeshi women, whether migrants or sedentees, were more likely to have experienced a hot flush in the last 2 weeks than Londoners of European origin. Bangladeshi women also experienced more frequent hot flushes in all 5 multiple regression models. Age at menopause and symptoms of the menopause are known to vary according to ethnicity [9, 25-27]. Murphy et al. [23] previously found that Bangladeshi women in this sample had an earlier age at menopause compared to European women, while Begum et al. [24] found that Bangladeshi sedentees and adult migrants had levels of hormones that confirmed an earlier process of reproductive ageing and an earlier decline in ovarian reserve. The significant differences between ethnic groups in their experience of hot flushes in the past 2 weeks may therefore be a reflection of variation in their relative ages at the menopausal transition.

A higher BMI significantly increased the probability of the occurrence of hot flushes in 3 of the 5 logistic regression models for all women, and was associated with an increased frequency of hot flushes in all 5 multiple regression models. A similar relationship with BMI has been found previously in some [38,45], but not all studies [46]. Aromatization of oestrogens occurs in adipose tissue and contributes to circulating levels [47]. These higher levels should theoretically be protective against menopausal symptoms, but the opposite effect is often observed. One explanation for the relationship between high BMI and increased incidence of vasomotor symptoms is Freedman's [48] suggestion that women with a greater BMI have increased body insulation and a narrower thermoneutral zone, making it easier for heavier women to suffer from hot flushes. Women with a high BMI might also be hotter from carrying around more weight, or may have a lower socioeconomic status (SES) or other characteristics predisposing them to vasomotor symptoms [46].

A higher education level lowered the probability of hot flush occurrence in 2 of the 5 logistic regression models, and was associated with less frequent hot flushes in all 5 multiple regression models. Education level is a good marker of SES, and women of lower SES experience an earlier menopause [49,50] and more vasomotor symptoms than women of higher SES [8]. Anxiety has been shown to increase the incidence and severity of hot flushes [28], and the thermoneutral zone may be narrowed due to elevated sympathetic activity [11]. An increased burden from infectious diseases during childhood has previously been shown to be significantly associated with a lower age at menopause among Bangladeshi sedentees and adult migrants [23], but was not significantly associated with hot flush occurrence or frequency in the analyses undertaken here. Finally, the frequency of hot flushes was also associated with menopausal status, such that peri- and post-menopausal women suffered hot flushes more frequently.

A limitation of this study is the small sample size, particularly with regard to hot flash frequency. This restricts the generalizability of the study results; however, the finding of a relationship between levels of AMH and hot flashes supports the use of AMH as a useful marker to be applied in future studies of hot flash etiology. A well-powered prospective study is needed to confirm this finding.

Conclusions

Relationships were found between AMH, FSH, and menopausal symptoms. Ethnicity, education level, and BMI were also found to be associated with the experience of vasomotor symptoms. Previous evidence suggests that age at menopause and the frequency and severity of hot flushes experienced during the menopausal transition vary between women and groups of women depending on a number of factors. This study was conducted to understand better the potential relationship between hormone levels and hot flushes among women from different ethnic groups that could explain this inter-individual and inter-group variation. Significant relationships were found between AMH, FSH and hot flush occurrence in the past 2 weeks. The decline of AMH and the rise of FSH are clinical markers of transition through the menopause. The relationships found in this study may be indicative of women progressing through the menopausal transition and so experiencing symptoms, rather than AMH and FSH being mechanistically involved in the generation of symptoms. It is of clinical relevance that, despite this study aiming to examine the specific relationships between reproductive hormones and symptoms of the menopause, lifestyle and ethnicity were also found to have an influence on the experience of menopause. This merits further research, and encourages a holistic approach to the management of menopause.

Acknowledgments: Funding for this study was provided by NSF grant #0548393, Commonwealth Scholarship and Fellowship Plan (CSFP, UK), Sigma Xi, and the Wolfson Research Institute, Durham University. Manuscript writing was supported by the Institute for Advanced Study, Durham University. We thank all the women who participated in the study and particularly the Bengali Women's Health Project for assistance. We are also thankful for the help of Lorna Murphy, Susmita Paul Jamy, Sunali Biswas, Nusrath Jahan, Mahbuba Bari Shikder, Shamima Begum, Sayema Islam Shetu, Sufia Sultana, Rumana Begum, Kanij Fathema, Salma Begum, Khaza Nazim Uddin, and Tamanna Sultana.

REFERENCES CITED

[1] Lee, S. J., Lenton, E. A., Sexton, L., Cooke, I. D, The effect of age on the cyclical patterns of plasma LH, FSH, oestradiol and progesterone in women with regular menstrual cycles. Human Reproduction. 3 (1988) 851-855.

[2] Rannevik, G., Jeppsson, S., Johnell, O., Bjerre, B., Laurell-Borulf, Y., Svanberg, L, A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas. 21 (1995) 103-113.

[3] Santoro, N., Randolph, J.F, Reproductive hormones and the menopause transition. Obstet Gynecol Clin North Am. 38 (2011) 455-466.

[4] Takahashi, T.A., Johnson, K.M, Menopause. Med Clin North Am. 99 (2015) 521-534.

[5] Grisendi, V., Spada, E., Argento, C., Plebani, M., Milani, S., Seracchioli, R., Volpe, A., La Marca, A, Age-specific reference values for serum FSH and estradiol levels throughout the reproductive period. Gynecol Endocrinol. 30 (2014) 451-455.

[6] Lie Fong, S., Visser, J.A., Welt, C.K., de Rijke, Y.B., Eijkemans, M.J., Broekmans, F.J., Roes, E.M., Peters, W.H., Hokken-Koelega, A.C., Fauser, B.C., Themmen, A.P., de Jong, F.H., Schipper, I., Laven, J.S, Serum anti-müllerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. J Clin Endocrinol Metab. 97 (2012) 4650-4655.

[7] Lipson, S.F., Ellison, P.T. Normative study of age variation in salivary progesterone profiles. J Biosoc Sci. 24 (1992) 233-244.

[8] Sievert, L.L, Menopause: A Biocultural Perspective. Rutgers University Press, 2006.

[9] Gold, E., Colvin, A., Avis, N., Bromberger, J., Greendale, G.A., Powell, L., Sternfeld, B., Matthews, K, Longitudinal analysis of vasomotor symptoms and race/ethnicity across the menopausal transition: Study of Women's Health Across the Nation (SWAN). Am J Public Health 96 (2006) 1226-1235.

[10] Hunter, M.S., Gentry-Maharaj, A., Ryan, A., Burnell, M., Lanceley, A., Fraser, L., Jacobs, I., Menon, U, Prevalence, frequency and problem rating of hot flushes persist in older postmenopausal women: impact of age, body mass index, hysterectomy, hormone therapy use, lifestyle and mood in a cross-sectional cohort study of 10,418 British women aged 54-65. BJOG 119 (2012) 40-50.

[11] Freedman, R.R, Pathophysiology and treatment of menopausal hot flashes. Semin Reprod Med. 23 (2005) 117-125.

[12] Archer, D.F., Sturdee, D.W., Baber, R., de Villiers, T.J., Pines, A., Freedman, R.R., Gompel, A., Hickey, M., Hunter, M.S., Lobo, R.A., Lumsden, M.A., MacLennan, A.H., Maki, P.,

Palacios, S., Shah, D., Villaseca, P., Warren, M, Menopausal hot flushes and night sweats: where are we now? Climacteric 14 (2011) 515-528.

[13] Freedman, R.R., Krell, W. Reduced thermoregulatory null zone in postmenopausal women with hot flashes. Am J Obstet Gynecol 181 (1999) 66-70.

[14] Freedman, R.R, Physiology of hot flashes. Am J Hum Biol 13 (2001) 453-464.

[15] Freedman, R.R, Menopausal hot flashes: mechanisms, endocrinology, treatment. J Steroid Biochem Mol Biol. 142 (2014) 115-120.

[16] Rance, N.E., Dacks, P.A., Mittleman-Smith, M.A., Romanovsky, A.A., Krajewski-Hall, S.J, Modulation of body temperature and LH secretion by hypothalamic KNDy (kisspeptin, neurokinin B and dynorphin) neurons: A novel hypothesis on the mechanisms of hot flushes. Front Neuroendocrinol. 34 (2013) 211-227.

[17] Henderson, K., Bernstein, L., Henderson, B., Kolonel, L., Pike, M.C, Predictors of the timing of natural menopause in the multiethnic cohort study. Am J Epidemiol 167 (2008) 1287-1294.

[18] Thomas, F., Renaud, F., Benefice, E., De Meeus, T., Guegan, J-F, International variability of ages at menarche and menopause: patterns and main determinants. Hum Biol 73 (2001) 271-290.

[19] Sievert, L.L, Anthropology and the study of menopause: evolutionary, developmental, and comparative perspectives. Menopause 21 (2014) 1151-1159.

[20] Kaczmarek, M., Pacholska-Bogalska, J., Kwaśniewski, W., Kotarski, J., Halerz-Nowakowska, B., Goździka-Józefiak, A, A microsatellite polymorphism in IGF1 gene promoter and timing of natural menopause in Caucasian women. Int J Med Sci. 12 (2015) 32-41.

[21] Markatseli, A.E., Hatzi, E., Pamporaki, C., Bouba, I., Xita, N., Tigas, S., Georgiou, I., Tsatsoulis, A, Association of the (TAAAA)n repeat polymorphism of SHBG gene with the age at menopause in Greek postmenopausal women. Maturitas 78 (2014) 113-116.

[22] Stolk, L., Perry, J.R., Chasman, D.I., He, C., Mangino, M., Sulem, P., Barbalic, M., Broer, L., Byrne, E.M., et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nat Genet. 44 (2012) 260-268.

[23] Murphy, L., Sievert, L.L., Begum, K., Sharmeen, T., Chowdhury, O., Muttukrishna, S., Bentley, G.R. Life course effects on age at menopause among Bangladeshi sedentees and migrants to the UK. *Am J Human Biology* 25 (2013) 83-93.

[24] Begum, K., Muttukrishna, S., Sievert, L.L., Sharmeen, T., Murphy, L., Chowdhury, O., Kasim, A., Gunu, R., Bentley, G.R. Ethnicity or environment: Effects of migration on ovarian reserve among Bangladeshi women in the UK. Fert Steril (2015, Epub ahead of print).

[25] Freeman, E.W., Sherif, K. Prevalence of hot flushes and night sweats around the world: a systematic review. Climacteric 10 (2007) 197–214.

[26] Obermeyer, C.M, Menopause across cultures: a review of the evidence. Menopause 7 (2000) 184–192.

[27] Melby, M.K., Sievert, L.L., Anderson, D., Obermeyer, C.M. Overview of methods used in cross-cultural comparisons of menopausal symptoms and their determinants: Guidelines for strengthening the reporting of menopause and aging (STROMA) Studies. Maturitas 70 (2011) 99-109.

[28] Freeman, E.W., Sammuel, M.D., Lin, H., Liu, Z., Gracia, C.R, Duration of menopausal hot flushes and associated risk factors. 117 (2011) 1095-1104.

[29] Green, R., Santoro, N. Menopausal symptoms and ethnicity: the Study of Women's Health Across the Nation. Womens Health (Lond Engl). 5 (2009) 127-1 33.

[30] Im, E.O., Lee, B., Chee, W., Brown, A., Dormire, S, Menopausal symptoms among four major ethnic groups in the United States. West J Nurs Res 32 (2010) 540-565

[31] Sievert, L.L., Begum, K., Sharmeen, T., Chowdhury, O., Muttukrishna, S., Bentley, G, Patterns of occurrence and concordance between subjective and objective hot flashes among Muslim and Hindu women in Sylhet, Bangladesh. Am J Human Biology 20 (2008) 598-604.

[32] Sharmeen, T., Sievert, L.L., Begum, K., Chowdhury, O., Muttukrishna, S., Bentley, G.R, Symptoms at midlife, family structure, and household workloads in Sylhet, Bangladesh. Anthropological Review 76 (2013) 199-216.

[33] World Health Organization (WHO). Research on the Menopause in the 1990s. WHO Technical Report Series No. 866, Geneva: WHO. 1996.

[34] Bentley, G.R., Muttukrishna, S. Potential use of biomarkers for analyzing inter-population and cross-cultural variability in reproductive aging. *Menopause* 14 (2007) 668-79.

[35] LaMarca, A., Grisendi, V., Griesinger, G. How much does AMH really vary in normal women? International Journal of Endocrinology (2013) 2013:959487.

[36] Freeman, E.W., Sammel, M.D., Lin, H., Gracia, C.R., Pien, G.W., Nelson, D.B., Sheng, L, Symptoms associated with menopausal transition and reproductive hormones in midlife women. Obstet Gynecol 111 (2007) 230-240.

[37] Freeman, E.W., Sammel, M.D., Lin, H, Temporal associations of hot flashes and depression in the transition to menopause. Menopause 16 (2009) 728-734.

[38] Gold, E.B., Block, G., Crawford, S., Lachance, L., FitzGerald, G., Miracle, H., Sherman, S, Lifestyle and demographic factors in relation to vasomotor symptoms: baseline results from the Study of Women's Health Across the Nation, Am J Epidemiol. 159 (2004) 1189-1199.

[39] Randolph, J.F. Jr, Sowers, M., Bondarenko, I., Gold, E.B., Greendale, G.A., Bromberger, J.T., Brockwell, S.E., Matthews, K.A, The relationship of longitudinal change in reproductive hormones and vasomotor symptoms during the menopausal transition. J Clin Endocrinol Metab 90 (2005) 6106-6112.

[40] Anderson, R.A., Nelson, S.M., Wallace, W.H, Measuring anti-Mullerian hormone for the assessment of ovarian reserve: when and for whom is it indicated? Maturitas 71 (2012) 28-33.

[41] Broer, S.L., Eijkemans, M.J., Scheffer, G.J., van Rooij, I.A., de Vet, A., Themmen, A.P., Laven, J.S., de Jong, F.H., Te Velde, E.R., Fauser, B.C., Broekmans, F.J, Anti-Mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women, J Clin Endocrinol Metab. 96 (2011) 2532-9.

[42] Tehrani, F.R., Solaymani-Dodaran, M., Azizi, F, A single test of antimulluerian hormone in late reproductive-aged women is a good predictor of menopause. Menopause. 16 (2009) 797-802.

[43] Newson, L, Menopause and its management. 2013. [online] Available: http//www.patient.co.uk/doctor/menopause-and-it-management [27/08/2014]

[44] Harlow, S.D., Gass, M., Hall, J.E., Lobo, R., Maki, P., Rebar, R.W., Sherman, S., Sluss, P.M., de Villiers, T.J.; STRAW 10 Collaborative Group. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging, Menopause. 19 (2012) 387-395.

[45] Office of Research on Women's Health. Highlights of women's health research for ORWH 20th Anniversary; focus on the menopause transition. (2010) NIH Publication No. 10-7606-D.

[46] Gallicchio, L., Miller, S.R., Kiefer, J., Greene, T., Zacur, H.A., Flaws, J.A. Change in body mass index, weight, and hot flashes: a longitudinal analysis from the midlife women's health study. J Womens Health (Larchmt). 23 (2014) 231-237.

[47] Nelson, L.R., Bulun, S.E, Estrogen production and action. J Am Acad Dermatol. 45 (2001) S116-124.

[48] Freedman, R.R, Hot flash trends and mechanisms. Menopause 9 (2002) 151-152.

[49] Sievert, L.L., Hautaniemi, S.I, Age at menopause in Puebla, Mexico. Human Biology, 75 (2003) 205-226.

[50] Hardy, R., Kuh, D, Social and environmental conditions across the life course and age at menopause in a British birth cohort study. BJOG, 112 (2005) 346-354.

Conflicts of interest: None.

Authors' roles: TD, LS, and GB interpreted the data, drafted the manuscript, and analyzed some of the data. GB, SM, and LS designed the study, directed implementation, and data collection. KB, LS, and TS collected the data. SM directed hormonal analyses, and assisted with data interpretation. AK assisted with data analysis and provided critical comments. OC provided logistical and laboratory support in Sylhet, Bangladesh.

Table 1: Descriptive statistics for all groups of women (n=108).

	Europeans	Adult Migrants	Sedentees	All women
No. of women	41	39	28	108
Mean age (sd)	49.3(5.8)	48.3(5.3)	49.4(5.7)	49.0(5.57)
No. aged 40-49 years (%)	22 (54)	24 (61)	12 (43)	58 (54)
No. aged 50-59 years (%)	19 (46)	15 (39)	16 (57)	50 (46)
Menopausal status				
No. pre-menopausal (%)	22 (54)	18 (37)	9 (32)	49 (45)
No. peri and post-menopausal (%)	19 (46)	21 (63)	19 (68)	59 (55)
Mean BMI (sd)	25.6 (5.2)	26.8 (3.1)	25.1 (5.2)	25.9 (4.6)
Relative level of education				
Low (%)	10 (24)	7 (23)	8 (29)	25 (25)
Medium (%)	15 (37)	8 (26)	3 (11)	26 (26)
High(%)	16 (39)	16 (52)	17 (61)	49 (49)
Exposures to infectious disease				
0-2 diseases (%)	14 (34)	21 (54)	15 (54)	50 (46)
3-4 diseases (%)	22 (54)	17 (44)	9 (32)	48 (45)
5-6 diseases (%)	5 (12)	1 (3)	4 (14)	10 (9)
No. (%) of women with hot flashes in past 2 weeks	11 (27)	23 (59)	16 (57)	50 (46)
Mean logInhibin B (sd)*	**1.3(0.33)**	**1.2(0.3)**	**1.1(0.2)**	**1.2(0.3)**
Mean logAMH (sd)	0.11(0.7)	-0.08(0.5)	-0.13(0.5)	-0.02(0.6)
Mean logFSH (sd)	1.4(0.5)	1.5(0.4)	1.56(0.47)	1.45(0.47)
Mean log E_2 (sd)	1.6(0.52)	1.5(0.4)	1.53(0.33)	1.48(0.42)

*p<0.05

Table 2. Results of logistic regressions comparing occurrence of hot flushes in the past 2 weeks by hormones, n=100.[1]

		Exp(B)	*95% confidence interval*	p-value
All hormones[2] Model 1	BMI	**1.13**	**1.01-1.25**	**0.03**
	Peri/post-menopausal status	0.29	0.05-1.86	0.19
	ETHNICITY			
	European (ref)			
	Adult migrant	**4.42**	**1.35-14.52**	**0.01**
	Sedentee	**5.15**	**1.17-22.73**	**0.03**
	EDUCATION LEVEL			
	Low (ref)			
	Medium	0.32	0.08-1.35	0.12
	High	**0.24**	**0.07-0.84**	**0.03**
	INFECTIOUS DISEASE LOAD			
	0-2 diseases (ref)			
	3-4 diseases	0.85	0.30-2.46	0.77
	5-6 diseases	1.53	0.30-7.72	0.61
	ALL HORMONES			
	LoginhibinB	5.22	0.24-113.76	0.29
	LogAMH	**0.14**	**0.02-0.92**	**0.04**
	LogFSH	4.11	0.39-43.02	0.24
	LogE_2	0.80	0.11-5.95	0.83
Inhibin B[3] Model 2	BMI	1.10	1.00-1.21	0.05
	Peri/post-menopausal status	1.81	0.60-5.48	0.29
	ETHNICITY			
	European (ref)			
	Adult migrant	**4.00**	**1.31-12.20**	**0.02**
	Sedentee	3.22	0.96-10.77	0.06

	EDUCATION LEVEL			
	Low (ref)			
	Medium	0.35	0.09-1.31	0.12
	High	0.37	0.12-1.11	0.08
	INFECTIOUS DISEASE LOAD			
	0-2 diseases (ref)			
	3-4 diseases	1.03	0.39-2.74	0.95
	5-6 diseases	1.25	0.29-5.36	0.76
	LoginhibinB	0.33	0.05-2.35	0.27
AMH[4] Model 3	BMI	**1.12**	**1.01-1.24**	**0.03**
	Peri/post-menopausal status	0.71	0.18-2.76	0.62
	ETHNICITY			
	European (ref)			
	Adult migrant	**4.07**	**1.31-12.67**	**0.02**
	Sedentee	**3.89**	**1.15-13.17**	**0.03**
	EDUCATION LEVEL			
	Low (ref)			
	Medium	0.28	0.07-1.14	0.08
	High education	**0.26**	**0.08-0.86**	**0.03**
	INFECTIOUS DISEASE LOAD			
	0-2 diseases (ref)			
	3-4 diseases	0.98	0.36-2.70	0.97
	5-6 diseases	1.59	0.33-7.61	0.56
	LogAMH	**0.19**	**0.05-0.69**	**0.01**
FSH[5] Model 4	BMI	**1.11**	**1.00-1.23**	**0.04**
	Peri/post-menopausal status	0.48	0.84-2.79	0.42
	ETHNICITY			

	European (ref)			
	Adult migrant	**4.13**	**1.32-12.92**	**0.02**
	Sedentee	**3.78**	**1.14-12.54**	**0.03**
	EDUCATION LEVEL			
	Low (ref)			
	Medium	0.38	0.10-1.46	0.16
	High	0.36	0.12-1.11	0.08
	INFECTIOUS DISEASE LOAD			
	0-2 diseases (ref)			
	3-4 diseases	0.81	0.29-2.26	0.68
	5-6 diseases	1.18	0.27-5.21	0.82
	LogFSH	**8.47**	**1.28-56.29**	**0.03**
E_2[6] Model 5	BMI	1.10	1.00-1.21	0.06
	Peri/post-menopausal status	1.79	0.52-6.12	0.36
	ETHNICITY			
	European (ref)			
	Adult migrant	**4.33**	**1.42-13.19**	**0.01**
	Sedentee	**4.37**	**1.28-14.87**	**0.02**
	EDUCATION LEVEL			
	Low (ref)			
	Medium	0.39	0.11-1.42	0.15
	High	**0.38**	**0.13-1.14**	**0.08**
	INFECTIOUS DISEASE LOAD			
	0-2 diseases (ref)			
	3-4 diseases	1.07	0.40-2.82	0.90
	5-6 diseases	1.08	0.25-4.68	0.92
	Log E_2	0.52	0.11-2.40	0.41

[1] Information was missing for 8 women so only 100 women are included in the logistic regressions conducted to predict the occurrence of hot flushes

[2] Model 1 (All hormones) significance: $X^2(12) = 32.11$, $p<0.01$, $NR^2=0.37$

[3] Model 2 (Inhibin B) significance: $X^2(9) = 24.07$, $p<0.01$, $NR^2=0.29$

[4] Model 3 (AMH) significance: $X^2(9) = 30.17$, $p<0.01$, $NR^2=0.35$
[5] Model 4 (FSH) significance: $X^2(9) = 28.29$, $p<0.01$, $NR^2=0.33$
[6] Model 5 (E2) significance: $X^2(9) = 22.50$, $p<0.01$, $NR^2=0.27$

Table 3: Multiple regression results predicting hot flush frequency in all women (n=62)[1]

			95% confidence interval	
		Exp(B)		p-value
All Hormones* Model A	BMI	**0.43**	**0.15-0.44**	**0.00**
	Menopausal status	**0.39**	**0.20-4.40**	**0.03**
	Ethnicity	**0.29**	**0.22-1.94**	**0.02**
	Education level	**-0.26**	**-1.70- -0.16**	**0.02**
	Infectious disease	-0.08	-1.37-0.60	0.43
	LogInhibinB	0.32	-0.44-6.89	0.08
	LogAMH	-0.13	-2.99-1.48	0.50
	LogFSH	-0.08	-3.29-2.31	0.73
	Log E_2	-0.07	-3.14-2.06	0.68
Inhibin B* Model B	BMI	**0.44**	**0.16-0.44**	**0.00**
	Menopausal status	**0.42**	**1.01-3.93**	**0.00**
	Ethnicity	**0.27**	**0.23-1.83**	**0.01**
	Education level	**-0.24**	**-1.59- -0.14**	**0.02**
	Infectious disease	-0.08	-1.33-0.57	0.43
	LoginhibinB	0.24	-0.23-5.05	0.07
AMH* Model C	BMI	**0.42**	**0.14-0.42**	**0.04**
	Menopausal status	**0.35**	**0.45-3.65**	**0.01**
	Ethnicity	**0.24**	**0.11-1.72**	**0.03**
	Education level	**-0.22**	**-1.52- -0.02**	**0.04**
	Infectious disease	-0.07	-1.30-0.66	0.51
	LogAMH	0.09	-1.11-2.14	0.53
FSH* Model D	BMI	**0.41**	**0.14.0.42**	**0.00**
	Menopausal status	**0.38**	**0.20-4.23**	**0.03**
	Ethnicity	**0.24**	**0.09-1.69**	**0.03**
	Education level	**-0.23**	**-1.55- -0.06**	**0.03**
	Infectious disease	-0.06	-1.3-0.68	0.55
	LogFSH	-0.10	-2.9-1.55	0.54
E_2* Model E	BMI	**0.42**	**0.14-0.42**	**0.01**
	Menopausal status	**0.34**	**0.28-3.72**	**0.02**
	Ethnicity	**0.22**	**0.01-1.66**	**0.05**
	Education level	**-0.23**	**-1.57- -0.08**	**0.03**
	Infectious disease	-0.06	-1.25-3.72	0.58
	Log E_2	0.07	-1.65-2.65	0.64

[1] Information on hot flash frequency was collected only from women who reported hot flashes, therefore only 66 cases are included in the multiple regressions conducted to predict hot flush frequency.

*Model 1 (All hormones) significance: $F(9,52) = 4.77$, $p<0.01$, $R^2=0.36$; Model 2 (Inhibin B) significance: $F(6,55) = 7.40$, $p<0.01$, $R^2=0.39$; Model 3 (AMH) significance: $F(6,55) = 6.56$, $p<0.01$, $R^2=0.35$, $NR^2=0.35$; Model 4 (FSH) significance: $F(6,55) = 6.56$, $p<0.01$, $R^2=0.35$; Model 5 (E2) significance: $F(6,55) = 6.50$, $p<0.01$, $R^2=0.35$

Figure 1. Percentage of women reporting hot flushes as "not at all," "a little," "quite a bit," or "extremely" during the past two weeks by group